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Seasonal variation of metal contamination in the barnacles *Pollicipes pollicipes* in northwest coast of Portugal show clear correlation with levels in the surrounding water

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ABSTRACT

The concentrations of metals were determined in northwest (NW) coast of Portugal seawaters and soft tissues of goose barnacles *Pollicipes pollicipes*. *P. pollicipes* can be used for monitoring metal contamination in these coastal seawaters, because there were significant correlations ($p < 0.05$) for all metals between soft tissues and seawaters during the four seasons. Metal concentrations in seawaters and *P. pollicipes* had significant ($p < 0.05$) spatial and seasonal variations and mean log BAFs for Fe and Cd were higher than for Cr, Cu, Mn and Zn. Regarding the metal concentrations obtained in the coastal seawaters, all NW coast of Portugal should be classified as "Class IV – Bad", except two locations (location 7 at Summer and location 10 at Winter), which should be classified as "Class III – Moderate". However, considering the metal concentrations bioaccumulated in *P. pollicipes*, all locations should be classified as "Class III – Remarkably Polluted" during all seasons of 2011.

The Water Directive is a Framework for the European Community Action in the Field of Water Policy. It requires the establishment of biomonitoring programs of aquatic ecosystems to assess their ecological quality and settles ecological goals, specific for each type of superficial water (Cortes and Oliveira, 2000). Several species are being used worldwide in Biomonitoring Watch Programmes such as in the United States of America, IndoPacific or Europe, using mussels – *Mytilus* and barnacles – *Amphibalanus* or *Balanus* (Lauenstein et al., 1990; Beliaeff et al., 1998; Cantillo, 1998; Rainbow and Blackmore, 2001; Rainbow et al., 2000, 2002, 2004; Reis at al., 2011). Philips (1977) suggested that biomonitor species should show significant correlations between the bioaccumulated and environmental concentrations in all locations, to avoid spurious ecological interpretations. In the particular case of Portugal, the actual Water Directive and European policies applied to assess the ecological quality of waters involve several parameters, which difficult their correct classification (Bordalo e Sá, 2001). The use of biotic indices and contaminants reference guidelines, such as Norwegian Pollution Control Authority SFT TA-2229/2007 (coastal seawaters) and SFT TA-1467/1997 (blue mussel *Mytilus edulis*) for metals, can be a useful solution, which allows an uniform ecological quality classification of coastal waters (Molvaer et al., 1997; SFT, 2007; Reis et al., 2012a,b).

Goose barnacles *Pollicipes pollicipes* were previously used by Reis et al. (2012b) to study metal contamination of these coastal waters but only during one season (Summer 2010). Thus, in this work, *P. pollicipes* was used again as biomonitor of metal contamination in coastal waters of the northwest (NW) coast of Portugal during one-year period (four sampling seasons). The main objectives were: (i) to assess the potential use of *P. pollicipes* as biomonitor of metal contamination in each season; (ii) to increase the information on metal concentrations in Portuguese seawaters and tissues of *P. pollicipes*; (iii) to assess spatial and seasonal variations of metal bioavailabilities; (iv) to establish relationships between metals in seawaters and *P. pollicipes* and (v) to calculate Bioaccumulation Factors (BAFs) of metals in *P. pollicipes*.

During the four seasons of 2011 (Winter; Spring; Summer and Autumn), 10 locations were selected and monitored along the NW coast of Portugal (Fig. 1). These locations showed consistent mega-populations of *P. pollicipes* during all year and are under different levels of metal contamination (Reis et al., 2012a,b).

The reagents were at least of pro analysis (p.a.) grade: HNO_3 (Fluka, 35% (w/w), Suprapure); resin Chelex-100 (Fluka, Na^+ form, 100–200 mesh, p.a.); NH_3 (Panreac, 25% (w/w), p.a.) and NH_4CH_3COO (Panreac, p.a.). The metal standard solutions were daily prepared by weight from stock solutions of 1000 mg L^{-1} (Fluka, p.a.)

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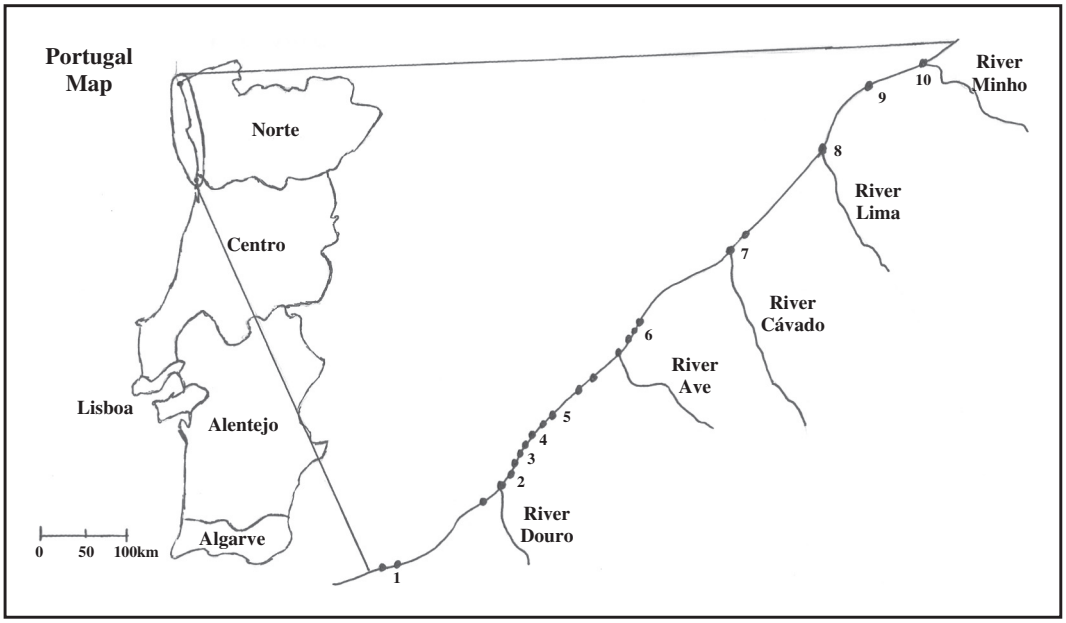

Fig. 1. Geographical distribution of the 10 sampling locations along the northwest coast of Portugal.

with ultra-pure water from Milli-Q system (conductivity: 0.054 μS cm^{-1} at 25 °C). Materials were pre-decontaminated in nitric acid solution (20%, v/v) and washed with deionised water (conductivity: <0.066 μS cm^{-1} at 25 °C; Elix-3 system), according APHA recommendations (APHA, 1998a).

In each sampling location and season, three independent replicates ($n = 3$) of coastal seawaters were collected with polyethylene bottles. In laboratory, seawater samples were filtered (Whatman, cellulose nitrate membrane filter, 0.45 μm), acidified (pH < 2) and frozen (−8 °C) until analyses (Reis et al., 2011b,c). Their dissolved metal concentrations (Cd, Cr, Cu, Fe, Mn and Zn) were analysed by Atomic Absorption Spectrometry (SpectrAA 220 FS, Varian) with flame atomization (Marck 7, Varian) or electrothermal atomization (Autosampler GTA 110, Varian) and with deuterium background correction system, according to APHA recommendations (APHA, 1998b,c). A solid phase extraction (SPE) method was applied to concentrate metals (concentration factor: 50) and remove interfering ions (Reis et al., 2012a,b). This SPE method was tested with seawater samples spiked with known amounts of each metal, which showed mean percentages of recovery higher than 93% for all elements (Cd: 105 ± 18%; Cr: 102 ± 17%; Cu: 111 ± 12%; Fe: 102 ± 22%; Mn: 93 ± 17% and Zn: 98 ± 18%).

Simultaneously with seawater, three independent replicates of *P. pollicipes* of at least 25 individuals (adult commercial-size: >2 cm, total length) were collected in each sampling location and season and transported in refrigerated plastic bags to laboratory within 8 h (Reis et al., 2012b). The peduncle muscle and entire body (soft tissues) of *P. pollicipes* were lyophilized, and homogenised (Rainbow et al., 2000; Rainbow and Blackmore, 2001; Morillo et al., 2005; Morillo and Usero 2008; Reis et al., 2012a,b). No differences among replicates ($p < 0.05$) in the mean dry weights of 25 pooled adult individuals were obtained, thus no significant effects of body size on accumulated metals are expected (Morillo et al., 2005; Morillo and Usero, 2008; Reis et al., 2012b). For metal analyses (Cd, Cr, Cu, Fe, Mn and Zn), soft tissues samples were digested following the method described by Reis and Almeida (2008). Total metal concentrations were determined by Atomic Absorption Spectrometry with flame atomization and electrothermal atomization, using the APHA recommendations (APHA, 1998b,c). The working parameters of the equipment and the matrix modifiers were those recommended by Varian Methods (Varian, 1988, 1989). Some samples were spiked with known amounts of metals, which allowed studying potential matrix effects in the determination of metals in soft tissues of *P. pollicipes*. These analyses showed mean recoveries above 95% for all elements (Cd: 98 ± 10%; Cr: 108 ± 18%; Cu: 102 ± 14%; Fe: 95 ± 13%; Mn: 106 ± 13% and Zn: 106 ± 7%). Finally, standard reference material certified for trace metals in mussel tissues (NIST SRM 2976) was used to study the suitability of the entire analytical procedure. These analyses showed mean recoveries above 90% for all elements (Cd: 90 ± 16%; Cr: 113 ± 23%; Cu: 97 ± 10%; Fe: 94 ± 14%; Mn: 102 ± 8% and Zn: 106 ± 3%).

Varian software was programmed to work with precisions below 10% between readings, in a maximum of four readings per replicate, to assure reproducibility of measurements. For each metal, external calibrations were carried out with aqueous standards solutions and blank solutions were prepared following the same treatment of samples. Metal concentrations in blanks were below the limit of detection of the analytical procedure for all elements in seawaters (Cd: 0.30 ng L^{-1}; Cr: 1.35 ng L^{-1}; Cu: 18 ng L^{-1}; Fe: 25 ng L^{-1}; Mn: 36 ng L^{-1} and Zn: 446 ng L^{-1}) and in soft tissues (Cd: 0.076 mg kg^{-1}; Cr: 0.035 mg kg^{-1}; Cu: 0.304 mg kg^{-1}; Fe: 7.99 mg kg^{-1}; Mn: 1.996 mg kg^{-1} and Zn: 26.6 mg kg^{-1}). The limits of detection were calculated using APHA recommendations (APHA, 1998d).

The statistical analyses of metal concentrations in seawaters and soft tissues of *P. pollicipes* consisted in a sequence of tests (Underwood, 1997; Rainbow et al., 2004; Silva et al., 2006): (i) normality of all data was checked with Shapiro–Wilk test, considering one level of significance ($p < 0.05$), using SPSS software; (ii) as some data failed normality test, all metal concentrations were logarithmically (base 10) transformed to create additive data sets with reduced deviations from normal distribution; (iii) homogeneity of variances was checked with Cochran test using WinGMAv 5 software (EICC, University of Sydney), considering one level of significance ($p < 0.05$); (iv) one-way analyses of variance (ANOVA) were used to identify significant differences ($p < 0.05$) in metal concentrations among locations (spatial variations) and seasons (seasonal variations), using WinGMAv 5 software (EICC, University of Sydney); (v) finally, unplanned comparisons (*post hoc* tests) were performed to establish groups of locations/seasons which